REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. CLAIM STATUS AND AMENDMENTS

Claims 1, 4, 5, 8-17, 20, 21 and 24-27 were pending in this application when last examined.

Claims 11 and 12 (in part) were examined on the merits and stand rejected.

Claims 1, 4, 5, 8-10, 13-17, 20, 21 and 24-27 were withdrawn as non-elected subject matter.

Claim 11 is cancelled without prejudice or disclaimer thereto in order to expedite allowance.

Claim 12 is amended into independent form. In amended claim 12, the term "granulation formation" is restricted to "granulation formulation in skin ulcer resulting from diabetes." Support for this amendment can be found on page 7, lines 3-5, and the working examples of the specification. Further, the phrase "(b) a protein comprising an amino acid sequence in which one to several amino acid(s) is/are deleted, substituted or added in SEQ ID NO: 1 of Sequence Listing, and having the HGF activity" is amended to any one of (b), (c), (d) and (e) as shown in the amended claim. The basis of this amendment is as follows:

(c) is supported by page 17, lines 14-18, of the specification; (d) is supported by page 17, lines 17-23, and page 17, line 26 to page 18, line 9, of the specification; and (e) is supported by page 18, lines 10-15, of the specification.

As for (b), there is a direct description about a nucleic acid having at least 95% homology with the nucleotide sequence described in SEQ ID NO: 2 and encoding a functional protein, i.e. a protein having HGF activity (page 18, lines 10-15 of the specification). If the nucleic acid sequence has at least 95% homology with SEQ ID NO: 2 and encodes a functional protein (a protein having HGF activity), it is naturally considered that the protein encoded by the nucleic acid has at least 95% homology to SEQ ID NO: 1 and encodes a functional protein. Therefore, part (b) is supported by the description on page 18, lines 10-15, of the specification.

Thus, no new matter has been added.

II. ENABLEMENT REJECTION

On page 2 of the Office Action, claims 11-12 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabled for the polypeptide of SEQ ID NO: 1, but not for other mutations (insertions, substitutions, or deletions) in the first Kringle or elsewhere within human HGF.

Applicants respectfully traverse this rejection as applied to amended claim 12. Applicants note that claim 12 now recites a greatly reduced genus of polypeptides including SEQ ID NO: 1, functional proteins having 95% homology to SEQ ID NO: 1, SEQ ID NO: 2, proteins which hybridize under highly stringent conditions to SEQ ID NO: 2 which are functional proteins, and functional proteins encoded by nucleic acid having 95% homology to SEQ ID NO: 2. Applicants further note that such members of the claimed genus are well-described in the specification. Thus, Applicants submit that the full scope of amended claim 12 can be practiced without undue experimentation. Thus, Applicants submit that this rejection, as applied to amended claim 12, is untenable and should be withdrawn.

III. INDEFINITENESS REJECTION

On page 3 of the Office Action, claim 12 was rejected under 35 U.S.C. § 112, second paragraph, as indefinite for dependency on non-elected claims. This rejection has been overcome, as applied to amended claim 12, for reasons which are self-evident.

IV. OBVIOUSNESS REJECTION

On pages 4-5 of the Office Action, claims 11-12 were rejected under 35 U.S.C. § 103(a) as being obvious over Toyoda et al. in view of Seki et al., Nakamura et al. (US 5,342,831), Nakamura et al. (EP 461,560) and Yoshida et al.

Applicants respectfully traverse this rejection as applied to amended claim 12.

The Office points out in the Office Action that Toyoda et al. discloses that over expression of HGF in transgenic mice promotes granulation. The Office further points out that Yoshida et al. discloses that inhibiting the action of HGF protein by using antibodies can suppress or inhibit granulation tissue formation.

However, the present invention recited in amended claim 12 is "a method for promoting granulation formation in skin ulcer resulting from diabetes". Both ulcers described in Toyoda et al. and Yoshida et al. are ulcers resulting from wound, not from diabetes. Please see "Materials and methods", "2.2 In vivo wound assay", page 95, line 42 of right column to page 96, line 2 of left column in Toyoda et al.; and page 336, lines 18-21 of the left column in Yoshida et al. Thus, Toyoda et al. and Yoshida et al. neither disclose nor suggest promoting granulation formation in ulcers resulting from diabetes.

Further, attached herewith is FDA guidance, i.e. "Guidance for industry: Chronic cutaneous ulcer and burn wounds-developing products for treatment", Wound Rep Reg 2001; 9: 258-268, which describes that "The claim (also referred to as the indication) refers not only to the beneficial effects of a product, as determined through clinical investigations, but also to the type of wound for which a product is intended (e.g., venous stasis ulcer, diabetic foot ulcer, pressure ulcer, burn sites, donor sites). Wounds differ pathophysiologically, making it difficult, - if not impossible- to generalize results obtained from a trial conducted in patients with one type of wound to those with another wound type. Separate safety and efficacy data will generally be necessary for each wound type for which an indication is sought." in the first paragraph of "II. CLAIMS", "A. General Considerations" on page 258 (Underline Added).

The above FDA guidance further describes that "Three of the major categories of chronic cutaneous ulcers are diabetic ulcers, venous stasis ulcers, and pressure ulcers. In general, separate trials are needed for each type of chronic ulcer because they have very different etiologies and potentially different response to therapy. The patient population chosen should be one that optimizes the study's ability to detect a treatment effect, but should also be a population that reflects the population for which the product will be indicated and used." in the first paragraph of "1. Chronic Cutaneous Ulcers" on page 263, right column (Underline Added).

Thus, it is clear from the above description by the FDA that the ulcers described in Toyoda et al. and Yoshida et al. are pathophysiologically quite different from the ulcer resulting from diabetes recited in amended claim 12. Therefore, even if Toyoda et al. discloses that over expression of HGF in transgenic mice promotes granulation and Yoshida et al. discloses that inhibiting the action of HGF protein by using antibodies can suppress or inhibit granulation

tissue formation, one of ordinary skill in the art cannot predict whether HGF can promote the granulation formation in skin ulcers resulting from diabetes.

Accordingly, Toyoda et al. and Yoshida et al. do not motivate a person of skill in the art to make the claimed invention, and thereby the claimed invention is not obvious form the cited references.

Further, it is described in Toyoda et al. that "the endogenous HGF is overexpressed in the HGF-overexpressing transgenic mouse" and further "VEGF is also induced in the HGF-overexpressing transgenic mouse". This reference further suggests that proliferation of endothelial cells in transgenic mice is due to the combined effect of HGF/SF itself and HGF/SF-induced VEGF. Toyoda et al. also suggests that enhanced fibroblast proliferation in transgenic granulation tissue is not a direct effect of HGF/SF (page 99, lines 16-23 of right column).

Such statements are explicitly teaching away, so that there is no expectation of success to administer only HGF to promote granulation formation in the skin ulcer resulting from diabetes.

The Office further points out that Nakamura et al. (US '831) discloses using HGF to treat skin and peptic ulcers. However, in Nakamura et al. (US '831), there is no description or suggestion regarding skin ulcers resulting from diabetes. Nakamura et al. (US '831) neither discloses nor suggests that the HGF variants recited in amended claim 12 promote granulation formation in skin ulcers resulting from diabetes. Therefore, one of ordinary skill in the art cannot expect the effect of the claimed invention from Nakamura et al. (US '831).

The Office further points out that one of ordinary skill in the art would have expected the HGF variant of SEQ ID NO: 1 to have this biological activity based on the teaching of Seki et al. and Nakamura et al. (EP '560).

However, as described above, Toyoda et al. and Yoshida et al. disclose ulcers resulting from wounds, which, as noted above, are different from ulcer resulting from diabetes. Further, as described above, Toyoda et al. explicitly discusses the effect of HGF on proliferation of endothelial cells and fibroblast, i.e. Toyoda et al. suggests that enhanced fibroblast proliferation in transgenic granulation tissue is not a direct effect of HGF (page 99, lines 16-23 of right column of Toyoda et al.). Therefore, even if one of ordinary skill in the art had knowledge of HGF variants and could combine Toyoda et al. and Yoshida et al., such artisan would not have

expected that such HGF variants promote granulation formation in skin ulcers resulting from diabetes.

The present inventors first found that, among HGFs, a human recombinant HGF with five amino acid residues deleted from the first Kringle domain is an excellent promoter of granulation formation in skin ulcers resulting from diabetes.

Such effect of the claimed invention is superior and unexpected in view of the cited prior art references to one of skill in the art. Such excellent effects of the claimed invention are well demonstrated by the working examples in the specification (page 37, line 24 to page 38, line 13 and page 40, lines 8-24 of the specification, and Fig. 2B).

Thus, for the above noted reasons, Applicants suggest that this rejection is untenable and should be withdrawn.

CONCLUSION

In view foregoing amendments and remarks, the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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ATTACHMENTS

1. FDA Clinical Focus Group. "Guidance for Industry: Chronic cutaneous ulcer and burn wounds-developing products for treatment." Wound Repair and Regeneration. July-August 2001, Vol. 9(4), pp. 258-268.

7091-28086

Guidance for industry: Chronic cutaneous ulcer and burn wounds—developing products for treatment

DRAFT—NOT FOR IMPLEMENTATION

FDA Wound Healing Clinical Focus Group[a]

I. INTRODUCTION

This document is intended to provide guidance[1] to sponsors on the development of drugs, biological products, and devices[2] to treat chronic cutaneous ulcer and burn wounds. The guidance contains recommendations about labeling claims, outcome measures, and trial design, as well as special considerations for preclinical development.

For the purposes of this guidance, a *chronic cutaneous ulcer* is defined as a wound that has failed to proceed through an orderly and timely series of events to produce a durable structural, functional, and cosmetic closure. This document specifically addresses venous stasis ulcers, diabetic foot ulcers, pressure ulcers, and burn wounds.

The Center for Biologics Evaluation and Research (CBER), the Center for Devices and Radiological Health (CDRH), and the Center for Drug Evaluation and Research (CDER) within the FDA regulate products to treat cutaneous wounds. This document contains guidance applicable to the development of products regulated by any of the three Centers. Center-specific issues and advice are noted where appropriate.

II. CLAIMS

A. General Considerations

The claim (also referred to as the *indication*) refers not only to the beneficial effects of a product, as determined through clinical investigations, but also to the type of wound for which a product is intended (e.g., venous stasis ulcer, diabetic foot ulcer, pressure ulcer, burn sites, donor sites). Wounds differ pathophysiologically, making it difficult — if not impossible — to generalize results obtained from a trial conducted in patients with one type of wound to those with another wound type. Separate safety and efficacy data will generally be necessary for each wound type for which an indication is sought.

Claims sought for the use of wound products should be prespecified before trials are performed and amenable to study using outcomes that are direct measures of clinical benefit or validated surrogates. The primary efficacy outcome is key to demonstrating the effectiveness of a product. In selecting endpoints, it is important to consider whether reliable means of assessing the endpoint exist, or can be developed.

Outcome measures for chronic cutaneous ulcers and burns are in evolution, as understanding of pathophysiology and techniques for wound treatment and assessment advance. Suggestions for possible outcome measures are based on the principles noted above and on the natural history and current management of burns and ulcers. Comments regarding other appropriate wound claims, endpoints, and assessment tools are invited.

Two broad categories of wound product claims include (1) claims related to improved wound healing and (2) claims related to improved wound care other than healing.

B. Claims Related to Improved Wound Healing

1. Incidence of Complete Wound Closure

A claim of *complete wound closure* for chronic, nonhealing wounds is considered the most clinically mean-

From the Food and Drug Administration's [a]Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, and Center for Devices and Radiological Health.

Reprint requests: No reprints will be available of this article. However it is accessible from the FDA web site, as noted in the preceding editorial.

ingful of the claims related to improved wound healing. *Complete closure* is defined as skin closure without drainage or dressing requirements. Generally, studies to support such a claim would be designed to measure incidence of complete wound closure in the treatment vs. the control groups by a specified time (landmark analysis). Efficacy *success* would be defined as a statistically significantly greater proportion of patients assigned active product achieving closure compared to the proportion in the control arm. The prespecified time for endpoint measurement should be based on the natural history of the disease process and the expected response to standard care.

The clinical benefit of wound closure that lasts for a very brief time is at best, highly limited. In general, trials should be designed such that subjects remain on study and continue to be evaluated at least 3 months following complete closure. The purpose for this follow-up period is to measure durability of the effect and to ensure that the product does not adversely affect durability of closure relative to standard care. For some products, durability of closure is also important for distinguishing wound healing from transient wound coverage.

Measurement of partial healing, if prospectively defined, may demonstrate relevant biological activity and be supportive of the determination of efficacy, but cannot be used as primary evidence of clinical efficacy. Partial healing, per se, is not considered an acceptable wound healing claim because the clinical benefit of statistically significant differences in wound size has not been established. Validated methods for measuring degrees of change in wound size also present difficulty. As described below, however, *partial healing that facilitates surgical closure* can be an acceptable claim.

2. *Accelerated Wound Closure*

A claim of *accelerated closure* reflects a clinically meaningful diminishing of the time until complete closure occurs. Time to event analysis (time to complete 100 percent closure) is recommended for this type of claim. A claim of *accelerated closure* should be supported by a finding of faster reduction in the size of the wound during the treatment period. Therefore, this claim requires accurate measuring of wound size over time.

For products that significantly increase the incidence of closure over the course of clinical study, the increased incidence of closure per se is likely to result in a superior outcome in rank analyses of time to healing, because even very slow healing counts as *faster* healing in such analyses as compared to failure to heal. Thus, the time to wound closure is most meaningfully compared when the incidence of complete closure is the same in both arms. As a result, given a finding of increased incidence of closure, the additional finding of superiority in time to complete closure may reflect little or no additional information about the product. When an improvement in time to closure results from an improvement in the incidence of closure, a claim of *improved incidence of closure* suffices to explain the clinical benefit and should not be supplemented by an additional claim of *accelerated wound closure.*

Accelerated healing claims for burns should distinguish between partial thickness burns, full thickness burns, or donor site wounds. *Accelerated closure* of the donor site produced during harvest of autologous grafts is a claim for which it is especially important to prespecify the clinical benefit expected because these partial thickness wounds heal well in 2 to 3 weeks with standard care regimens. For example, a product that accelerated healing of donor sites by only 1 or 2 days might provide clinical benefit if it could be safely used in extensively burned patients requiring repeated reharvesting of donor sites. If time to reharvest is used as the primary efficacy outcome to support this type of claim, careful attention to masking is important to prevent bias, since reharvest is generally undertaken before the donor site reaches 100 percent re-epithelialization.

Accelerated healing claims based on study of donor sites cannot be generalized to burns and chronic cutaneous ulcers because burns and ulcers do not share the clinical characteristics of uniform, partial thickness donor sites. However, for systemically administered test products, healing of both the donor sites and the ulcer or burn are important safety outcomes. For example, a product that accelerates the healing of donor sites should not worsen graft take.

3. *Facilitation of Surgical Closure*

The Agency does not consider partial healing per se to be an appropriate claim for wound healing agents because the clinical benefit of statistically significant decreases in wound size has not been established. However, agents that heal wounds to the point that surgical closure is more feasible, safer, or more effective may lead to the claim of *facilitates surgical closure.* Studies should be designed to measure the incidence of complete wound closure following application of the surgical graft. The durability and quality of surgical wound closure should be assessed over time to ensure that the product does not have a deleterious effect on these outcomes.

Timely excision and grafting have greatly reduced morbidity and mortality in patients with full thickness burns. The clinical benefits of engraftment in burn injury include reduced wound sepsis rates, improved hemodynamic status, and decreased requirement for donor site harvest. Since engraftment rates are high with good standard care, studies of surgical closure of burn wounds may take large numbers of patients to detect a difference between the test product and standard care. It is important to evaluate healing outcomes such as durability, functionality, and cosmetic appearance, including scarring.

4. *Improved Quality of Healing*

Trials for *improved cosmesis* claims should demonstrate a significant effect on outcomes such as scarring, the contour and feel of the healed skin, or normalization of skin markings or pigmentation. The appropriateness of an improved cosmesis claim depends on the type and location of the wound. For example, normalization of skin markings or pigmentation would clearly benefit patients who require grafting of full thickness burns on the face, whereas this outcome would be a less convincing measure of benefit for patients with plantar ulcers. In choosing endpoints to support improved cosmesis claims, it is important to consider whether a reliable assessment tool exists, or can be developed.

Products that reduce scarring may also improve function, for example, range of motion. Adequate assessment of this outcome requires standardization across treatment arms of use of concomitant therapies, such as pressure garments and rehabilitative therapies (e.g., passive range-of-motion exercises).

C. Other Considerations Related to Improved Wound Care

FDA recognizes that products intended for wound management may provide important patient benefit without improving the incidence or timing of closure relative to standard care. However, it is important to demonstrate that such products do not significantly impede healing. Thus, wound healing must be evaluated as a safety outcome for all products with a wound care claim.

1. *Wound Infection Control*

Infected wounds do not heal, and the primary efficacy outcome for topical anti-infective wound products can be either *healing* or *control of infection*. Both outcomes should be assessed, and reasonable concordance would be expected. Products for treatment or prophylaxis of infection in serious wounds (e.g., burns, diabetic foot ulcers) should have a well-established and appropriate spectrum of activity.[3]

2. *Debridement*

It is generally accepted that necrotic tissue inhibits healing by interfering with tissue repair and promoting microbial growth. Thorough debridement of wounds is therefore considered standard care essential to healing. *Partial debridement* is not an acceptable endpoint because the clinical benefit of partial debridement is unclear, and methods for measuring extent of debridement have not been validated. Although there is debate about the optimal design of trials to assess the efficacy of debriding agents, a reasonable endpoint for a debridement claim might be *thorough* removal of necrotic tissue (e.g., produces a wound bed suitable for grafting). Other clinically relevant endpoints, such as pain or blood loss during or immediately following debridement, could provide supportive evidence for clinical benefit when the primary efficacy endpoint is debridement equivalent to that produced by standard mechanical/surgical procedures. For burn wounds, **timeliness** of thorough debridement is an especially important consideration. Note that all studies should assess the debriding product's effects on wound closure to ensure that the product does not impair healing or cosmetic outcome.

3. *Wound Pain Control*

Studies of topical products that reduce wound site pain should distinguish between chronic wound pain and acute pain associated with wound care procedures. Appropriate instruments to measure pain should be prospectively defined and properly validated. The effect of topical pain control products on healing is an important safety outcome.

4. *Other Wound Care Claims*

Serious wounds may negatively affect many aspects of patients' lives. Clinically significant improvement in certain aspects of daily living not already captured by any of the previously described outcome measures (e.g., decreased drainage when experienced by the patient as an important improvement in ability to function) might support a labeling claim if demonstrated with a validated instrument.

III. PRECLINICAL CONSIDERATION

This section consists of specific points to consider for wound indication drugs and biological products. It is not intended as a general guidance for preclinical testing.[4]

A. Animal Models for Wounds

Wound models may be helpful in establishing pharmacological responses, as well as assessing potential toxicities of wound products. The animal species selected should exhibit a biological responsiveness to the test agent (i.e., should be a relevant species), where appropriate. Although animal models have been useful to establish proof of concept for some types of products, in general they have been poor predictors of efficacy in clinical trials. Because currently there are no ideal animal models for chronic wounds or extensive burns, multiple animal models are typically used to assess activity of wound healing agents. Fibroplasia and stroma formation can be evaluated by subcutaneous injection of some products. Contraction and re-epithelization can be evaluated by topical application on full thickness excisional wounds or in a pig graft donor site model. (Pigs are often useful models since their cutaneous architecture is most similar to that of human skin.) Induction of angiogenesis can be evaluated in chick chorioallantoic membrane or rabbit cornea. Breaking strength can be tested in a rat linear incision model. In impaired-healing models, the window of time for measuring treatment effects is extended. Impaired healing models include infection, necrotizing trauma, irradiation, administration of corticosteroids or chemotherapeutic drugs, or drug-induced or genetic diabetes mellitus in mice, rats, hamsters, guinea pigs, and young pigs. Each model has one or more of the characteristics that can be useful for evaluating a product's activity. For example, the rabbit ear dermal ulcer model lacks the vigorous wound contraction seen in rodent models and allows for the induction of ischemia in the wound.

B. Biodistribution and Pharmacokinetic Studies

In vivo biodistribution/pharmacokinetic studies are helpful in the design of toxicology studies. Preferably, the pharmacokinetic (PK) profile can be determined in the same animal species that will be used in the toxicity assessment. For topical wound products, animal wound models may provide more relevant information than application to intact animal skin. Since currently there are no chronic ulcer models, regional and/or systemic exposure after topical applications of a product for a chronic indication might be better approximated by subcutaneous injection (when technically feasible). Consideration should also be given to alterations of the PK profile and the potential for product accumulation with repeated dosing. Where feasible, information regarding the stability of the product at the target site, and for biological products, target receptor levels, contribute to a better understanding of the activity and potential toxicity of the wound product.

C. Toxicity Studies

The design of nonclinical toxicology studies for wound products should reflect, as much as possible, the intended clinical use of the product with respect to route, dosing regimen, and duration of exposure. It is important to assess any exaggerated pharmacological responses and potential toxicities of wound products. Administration of the wound product at multiples higher than the intended therapeutic dose (determined from wound models) may provide an estimate of the therapeutic index (toxic dose/effective dose) to aid in the selection of the initial clinical starting dose. Vehicle and sham controls should be employed where appropriate, to evaluate any adverse effects of product formulation components on wound healing.

Cutaneous irritation and hypersensitivity testing are generally indicated for all topically applied wound products, since these adverse reactions can seriously complicate human wounds. Products that will be delivered in an aerosol formulation should be evaluated for pulmonary toxicity, and possibly ocular toxicity (products known to be cutaneous irritants are assumed to be ocular irritants, and testing is generally waived).

The immunogenic potential of biotechnology-derived wound products can be a confounding factor in repeat dose toxicology studies because antibodies to the administered product may affect the PK profile, the pharmacodynamic response, and/or the toxicity of the agent. Although the development of antibodies to antigenic products has generally not been predictive of the clinical response, data on this should be collected to provide a complete preclinical safety assessment of the wound product.

Carcinogenicity studies generally should be conducted for drugs intended to treat chronic ulcers.[5] For biological products, the 2-year chronic bioassay and carcinogenicity study currently used for drugs is generally inappropriate due to species specificity and immunogenicity of the product. However, data in rodent initiation-promotion carcinogenesis models support the potential of various growth factors to act as tumor promoters. Current unresolved issues regarding the carcinogenic and tumorigenic potential of wound healing products include the likelihood of tumor promotion in the proposed patient populations and the additional susceptibility of patients exposed to environmental or other potential carcinogens (for example, systemic chemotherapy). Sponsors are en-

couraged to address this issue by referencing the existing scientific literature, and evaluating the potential of the test agent to stimulate the growth of normal and/or malignant cells that express the receptor for the agent.

Reproductive and developmental toxicology studies are recommended for wound products administered to women of child-bearing potential.[6]

Genotoxicity studies should be performed for all nonbiological drugs. These studies are indicated for a biotechnology-derived product only when supported by appropriate scientific rationale.[7]

IV. CLINICAL TRIAL CONSIDERATIONS

This section consists of specific points to consider for wound indication trials. It is not intended as general guidance on trial design.[8]

A. Absorption Studies

For topical drug, biological, and combination products, phase 1 evaluations should include quantitation of absorption through the wound. Systemic bioavailability of topically applied products is generally assessed using standard pharmacokinetic measurements with serial serum sampling. Systemic uptake is influenced by wound factors such as size and vascularity, as well as product characteristics such as molecular weight, chemical composition, and the presence of excipients. In the case of growth factors, relatively little (<1%) absorption typically occurs from chronic ulcer sites, but these amounts might be clinically significant because some growth factors are active in vitro at nanogram concentrations. For this reason, it is important to perform sensitive assays against serum background.

For products that are absorbed from the wound bed, the systemic dose depends on several factors: the concentration of the active ingredient, the total body surface area treated, the volume applied, frequency of application, and duration of contact with the wound.

Safety and pharmacokinetic studies for topical wound products should usually be conducted in patients with the indication sought, since absorption through intact skin of a normal volunteer would not predict absorption in a wound.

B. Irritancy or Sensitization

When preclinical studies or previous clinical experience suggest that a topical product might induce clinically significant dermatitis, irritancy or sensitivity testing in normal volunteers is recommended prior to trials in patients, since superimposed dermatitis is deleterious to wounds. The need for routine testing of the final formulation depends on the product, and sponsors are encouraged to discuss dermal toxicity testing with the appropriate Center before initiating the studies.

C. Assessment/Quantification

The tools to assess endpoints for a clinical trial should be both prespecified and standardized across clinical sites. For example, if photographs are to be used for measurement and documentation, the lighting and type of camera should be specified. Scoring systems for wounds can be used at baseline to determine eligibility for study, as well as for periodic wound assessment during the study. The use of accepted assessment systems is recommended (e.g., Wagner, International Association of Enterostomal Therapists). Proposals for novel assessment systems should include validation data.

Methodologies for quantifying wound characteristics are continually being developed, and sponsors are encouraged to discuss new approaches for their trials with the Agency. Regardless of the methodology, the following variables should be addressed in all clinical trials for wound indication products.

1. Ulcer Classification

The type of chronic ulcer (venous stasis, diabetic, pressure, arterial insufficiency) can usually be determined by considering the patient's history and performing a physical examination. Objective tools to confirm the diagnosis can include Doppler sonography to quantify venous or arterial insufficiency, transcutaneous oxygen tension ($tcpO_2$) measurements, ankle/brachial index, filament testing to quantify sensory neuropathy, measurement of laboratory markers for diabetes mellitus, and histopathology of ulcer biopsies to exclude neoplastic, immune-mediated, or primary infectious disease.

2. Wound Size

Quantitative measurements of wound size are routinely used to assess initial wound size before and after debridement, as well as progress toward closure. For ulcers that tend to be superficial, such as venous stasis ulcers, the area of the wound opening should be measured. This can be accomplished by tracing the wound perimeter or by measuring maximal width and length. For ulcers that extend deeply into tissue, volume or surface area should be measured when feasible. The extent of tissue undermining and sinus tracts is an important part of the evaluation. In the case of diabetic ulcers, qualitative assessment by probing the maximal depth is a frequently

used method. For other ulcers, such as pressure ulcers, molds can be used to provide precise measurement of volume and/or surface area. Alternatively, semi-quantitative measurements can be achieved using the maximal width/length/depth and shape coefficient.

For acute burns, it is important to determine as well as possible the depth of target burn wounds, as this parameter affects both the choice of standard of care regimen and the expected time to healing. The distinction between partial, full thickness, and indeterminate wounds is currently based on clinical judgment. Clinical parameters include appearance of the tissue, sensation, and bleeding upon debridement. Validated test methods for determining burn depth do not exist currently, but biopsy and Doppler measurement of blood flow are sometimes used. Wound depth heterogeneity is often an impediment to quantitative measures, and burn depth extension in the first 24 to 48 hours following injury frequently necessitates reassessment of wound severity and treatment. Initial clinical assessment of full thickness wounds should be confirmed by comparison to the total body surface area ultimately grafted.

When the target wound is an autograft donor site, the protocol should clearly delineate the method for harvest, and the size, thickness, and anatomic location of the donor site.

3. *Wound Imaging*

Photographic and wound imaging procedures standardized across all study sites should be used to document the wound appearance at each clinic visit and to corroborate the measurements captured in the case report form.

4. *Infection*

Infection should be assessed clinically by symptoms and signs that include purulent drainage, erythema, warmth, exudation, odor, pain, fever, and leukocytosis. Fever, pain, and leukocytosis may be absent, however, especially in patients with diabetic foot ulcers. Quantitative and qualitative culture of a viable tissue biopsy can be used at baseline to help determine if the wound is infected or merely colonized and to guide appropriate antimicrobial therapy. This method is generally preferred to quantitative and/or qualitative culture of swab specimens.[9]

D. Population

The choice of patient population for inclusion in clinical trials depends on the type of wound.

1. *Chronic Cutaneous Ulcers*

Three of the major categories of chronic cutaneous ulcers are diabetic ulcers, venous stasis ulcers, and pressure ulcers. In general, separate trials are needed for each type of chronic ulcer because they have very different etiologies and potentially different responses to therapy. The patient population chosen should be one that optimizes the study's ability to detect a treatment effect, but should also be a population that reflects the population for which the product will be indicated and used.

Variability can be reduced by specifying enrollment criteria that exclude conditions known to impede healing. For example, specifying a range for ulcer size will avoid ulcers that would be expected to close rapidly with little intervention (e.g., < 1 cm^2), and ulcers that would be less likely to close during a trial (e.g., > 50 cm^2). However, if demonstration of efficacy is limited to ulcers of a specific size, and the ability to extrapolate to smaller or larger ulcers is unclear, the labeled indication may be similarly limited.

2. *Burns*

The population for burn trials is usually defined by the extent and depth of the burn injury. For most burn wound claims, it is important to determine, to the extent possible, the depth of target wounds, since this determines the standard of care and the expected time to healing.

Important characteristics of the burn include its cause (thermal, chemical, electrical), anatomic location, depth (full or partial thickness), duration, and extent (% total body surface area). Patient characteristics that affect burn wound healing include age, nutritional status, underlying medical conditions, and the presence of concomitant injury (e.g., head trauma, inhalation injury, bone fractures). Patients with serious burns commonly receive multiple concomitant treatments, making it sometimes difficult to detect a treatment effect. For this reason, it is advisable to enroll patients with the least serious burns that still permit assessment of the product's claimed benefit. However, it may also be important to assess the effects of the study treatment used in conjunction with commonly used concomitant therapies.

When patients with full thickness burns are studied, donor sites for autografts are sometimes selected as the target wound. As noted earlier, although the patient population is one and the same, demonstrating the safety and efficacy of a product for a donor site wound does not support the safety and efficacy of the product for burn wounds, because burn wounds differ in clinically significant ways from surgical wounds.

E. Standard Care

Standard care in the context of this guidance refers to wound care in a clinical trial other than the experimental product. Good standard care procedures in a wound trial are a prerequisite for assessing safety and efficacy of a product. Since varying standard care procedures can confound the outcome of a clinical trial, it is generally advisable that all participating centers agree to use the same procedures. If standard care procedures are not uniform, it is important that the sample size and collected data be adequate to assess the impact of wound care variations on outcomes and treatment response.

A number of standard procedures for ulcer and burn care are widely accepted. The appropriate procedures to specify in clinical trials will evolve as care for wound and burn indications evolves. Several professional groups have initiated development of care guidelines for ulcers and burns. Although the Agency does not require adherence to any specific guidance, the basic guiding principle is that standard care regimens in wound trials should optimize conditions for healing and be prospectively defined in the protocol. The rationale for the standard care chosen should be included in the protocol, and the study plan should be of sufficient detail for consistent and uniform application across study centers. It is important to specify in the case report form (CRF), at each visit the type of ulcer or burn care actually delivered (for example, extent of debridement, use of concomitant medications). For outpatients, the CRF should also capture compliance with standard care measures, such as wound dressing, off-loading, and dietary intake. The value of study site consistency in standard care for reducing variation cannot be over-emphasized because of the profound effects these procedures have on clinical outcome for burns and chronic wounds. Nonetheless, in some cases it may be important to assess the effect of experimental treatment with common variations of standard care procedures.

1. *Standard Care Considerations for Chronic Cutaneous Ulcers*

Parameters for consideration in choosing standard care procedures for chronic cutaneous ulcer trials include the following.

- Removal of necrotic or infected tissue
- Off-loading of pressure and diabetic foot ulcers
- Compression therapy for venous stasis ulcers
- Establishment of adequate circulation for arterial ulcers
- Maintenance of a moist wound environment
- Infection control
- Nutritional support, including blood glucose control for diabetic ulcer patients
- Bowel and bladder care for patients with pressure ulcers at risk for contamination

a. Debridement

The presence of necrotic tissue, sinus tracts, exudation or transudation, and infection of soft and hard tissues can interfere with ulcer healing. Appropriate debridement procedures for the indicated ulcer should be specifically defined in the protocol. To avoid bias and confounding of treatment effect, ulcer debridement should precede evaluation of ulcer extent and infection. Enzymatic debriding agents, like other concomitant topical products, can confound results in wound product trials and generally should be avoided.

The need for additional debridement, performed after study treatment has started, may indicate product-induced wound deterioration. As such it should be documented on CRFs and included in analysis of product safety and efficacy. Discontinuation might be indicated in early trials where little is known about product safety, but not in later trials, when standard debridement procedures may be indicated to optimize patient care (e.g., on-going removal of callus as part of standard care for diabetic ulcers).

b. Off-loading/Compression

Relief of pressure is critical to outcome for chronic ulcers. Pressure is the principal cause of decubitus ulcers and off-loading is often difficult to standardize because equipment (e.g., type of bed) may not be available at all sites, and compliance with study procedures is labor intensive (e.g., turning). If these critical aspects of effective therapeutic intervention cannot be standardized across all sites, it is important to specify the actual care delivered in CRFs and to consider concomitant care in the efficacy analysis. For diabetic foot ulcers, off-loading choices (e.g., casting) must be weighed against the need to apply study treatments and monitor outcome. Similar considerations are important in choosing compression methods for venous stasis ulcers. Every attempt should be made to define a regimen that can be uniformly applied across sites and deviations should be captured in the CRFs.

c. Maintenance of a Moist Wound Environment

Maintenance of a moist wound environment is generally accepted standard care for all chronic cutaneous ulcers. In choosing test dosing regimens, it is helpful to consider limitations imposed by various standard care dressings. In cases where there is a sound rationale for the expected benefit of a test product, but its use is not compatible with established standard care dressings, alterations in standard care can usually be safely implemented by including adequate discontinuation rules.

d. Infection Control

Absence of frank infection is necessary for treatment success of all wound products, regardless of the claim. For this reason, wound products whose claim is not anti-infective are usually tested in patients with uninfected target ulcers (noting the distinction between colonization and frank infection of an ulcer). Acceptable ulcers for enrollment can often be achieved during a run-in period with thorough debridement and other good standard care procedures. A high incidence of true infection (as opposed to colonization) is present at baseline for diabetic foot ulcers. It may not always be necessary to exclude infected diabetic foot ulcers if the infection does not involve underlying structures and is responding to standard systemic anti-microbial therapy. In such cases, it is especially important that the protocol clearly delineate adequate rules for patient discontinuation due to wound deterioration on-study. As for all discontinued patients, safety assessment should continue throughout the trial and these patients should be included in efficacy analysis.

If an ulcer becomes infected during a study for a topical wound product, and the investigator prescribes topical anti-microbial treatment, it is recommended that the patient be discontinued from study treatment. Use of concomitant topical medication is discouraged in trials for topical products to avoid confounding of safety and efficacy outcomes.

Systemic antimicrobial therapy for target wound infection may become necessary during the treatment period of the study. Whether or not study treatment should be discontinued in this situation should be discussed prospectively and the plan included in the protocol. For example, discontinuation might be indicated in early trials, where little is known about product safety and where infection may signal test product-induced deterioration of the wound, but not in later trials where such therapy would be considered standard care (e.g., systemic antimicrobial therapy for diabetic ulcers).

e. Wound Cleansing

Agents used for wound cleansing should be bland (e.g., normal saline) because some cleansers retard healing, or can cause irritation and sensitization. The regimen should be prespecified in the protocol.

f. Nutritional Support

Caloric intake and metabolic status should be captured in the CRFs if the product is known to have metabolic effects (e.g., anabolic steroids). For products not known to have metabolic effects, these data may be useful if the inclusion criteria encompass patients significantly above or below ideal body weight (e.g., cachectic patients with pressure ulcers). Maintenance of normoglycemia is an important factor for patients with diabetic ulcers.

2. Standard Care Considerations for Burns

Standard care for serious burns includes careful attention to the following parameters.

- Hemodynamic resuscitation
- Management of comorbidities
- Timely burn debridement and/or excision
- Wound closure
- Infection control
- Pain control
- Nutritional support
- Rehabilitation, including passive range of motion when burns overly joints

Because large burn centers tend to have well-established, distinct standard care regimens, analysis of data in multicenter burn trials may require stratification by center. Since standard care procedures have profound effects on clinical outcome, every effort should be made to reach agreement among site investigators and to capture actual care delivered in the CRFs.

F. Safety Considerations

Specific points to consider for wound products are listed below.

1. Effects of the Product on the Wound

All wound treatment trials should include an evaluation of the product's effect on the healing process, as a safety outcome. Deterioration of target wounds can manifest as erythema, pain, discharge, infection, tissue necrosis, requirement for repeat debridement or other surgical intervention (i.e., amputation), and/or increase in ulcer size. Undesirable alterations of soft tissues, ligaments, periosteum, or joint capsules underlying deep wounds should also be evaluated, depending on the nature of the product. For detailed information about wound product microbiology, please see the attachments.

2. Immune Reactions

For biological products and some drugs, immunogenicity is generally addressed by measuring antibody titers prior to and after the treatment. Further immunologic characterization may be recommended, since the development of an immune response can render the product inactive (neutralizing antibodies), and/or induce acute or chronic immune reactions (e.g., anaphylaxis, contact sensitization, autoimmune disease).

3. Trial Stopping Rules

Because the patient populations in burn and chronic ulcer trials often have a high background incidence of serious adverse events, it is recommended that a safety data monitoring group be used for masked trials when the known or suspected risk is significant, and/or the study population is critically ill (e.g., seriously burned patients).

4. Patient Discontinuation

Discontinuation criteria evolve as the safety database for the wound product grows. Because the active ingredient(s) or the vehicle of topical wound products may exert a deleterious effect on healing, patients should be discontinued from study treatment if signs or symptoms suggest wound deterioration during early trials. Once reasonable assurance has been achieved that the product does not harm the wound, it may be appropriate to continue study treatment in later trials, depending on the claim and the type of wound. Subjects who are discontinued from study treatment should remain in the study for safety assessment and efficacy analysis.

G. Study Design Considerations

1. Randomization and Stratification

Randomization is particularly important to reduce bias in trials for wound indications because standard care wound management procedures and baseline wound characteristics have a profound effect on outcome. Because some degree of variation in these factors across patients and sites is unavoidable, stratification by study center is recommended to ensure balance between the arms. In some cases, it may be appropriate to prospectively stratify randomization by other important covariates, such as wound size or duration, but the number of variables used for stratification should be very limited. Variables thought to affect outcome should be considered in the analysis whether or not used for stratification (see Statistical Considerations).

2. Comparator Arms

A vehicle control arm is recommended for most wound product studies, with identical standard care procedures included in both the vehicle and investigational product arms. To evaluate the safety and effect of the vehicle, a study arm treated with standard care alone is recommended in phase 2 for topical wound products, if the safety of the vehicle has not been previously demonstrated.

Within patient control designs have been used in trials of topical products intended for serious burns, in an attempt to minimize the heterogeneity characteristic of this patient population. However, this approach compromises the evaluation of systemic toxicity, necessitating additional controls or studies to collect adequate safety data.

3. Masking

In general, masking (blinding) of patients and investigators to the treatment received will reduce bias and should be employed when feasible. Early studies of topical wound indication products often require an arm that receives only standard care, in addition to an arm receiving vehicle, to establish whether the vehicle has an effect on healing. Often the standard care only arm cannot be masked. In other cases, especially in some devices, it is impractical or unethical to implement a control treatment that mimics the test product and allows masking. In these types of situations, assessment by a third party masked evaluator should be considered.

H. Statistical Considerations Specific for Wound Product Trials

This section addresses issues that present special considerations for wound product trials.[10]

1. *Significance Tests*

Analysis should be prespecified in the protocol. For *incidence of closure* endpoints, categorical techniques are recommended (e.g., X^2 tests of homogeneity or logistic regression). For *time to closure* endpoints, outcome survival analyses are performed. For most wound trials, the center or investigator is almost always needed as a factor in the analysis, due to variations in standard of care. When appropriate, comparison of the survival curves can be done by using a Mantel-Haenszel statistic or by the Cox Proportional Hazards Model, which allows for covariate adjustment (including an adjustment for center). If rate of healing is being considered, growth curve models can be used to analyze the rate of healing.

2. *Missing Values and Imputation*

Missing values can affect the interpretation of a dataset, and for that reason steps should be taken to avoid them. When a substantial portion of values is missing, concerns arise about the adequacy of the trial. For that reason, a plan to account for missing values should be included in the protocol. The worst case outcome can be used to determine the maximal effect of missing values.

3. *Data Transformation and Covariate Analyses*

Prospective stratification should balance the arms for the one or two most important variables in the wound claim. Covariate analyses should be employed to adjust for variables that affect the outcome. These covariates should be prespecified, and the analyses should also be prespecified to avoid concerns about interpretability of significance tests.

When analyzing covariates, experience suggests that it is generally not useful to transform continuous variables into dichotomous variables (e.g., baseline ulcer size ≥ 5 cm^2 duration of the ulcer > 1 year). The covariate should be used as a continuous variable. Exploratory analyses may examine subgroups defined by various cut points, but when a particular cut point is deemed to be important in guiding the use of the product (e.g., ulcers greater than 10 cm do not respond), this cut point should be prospectively identified and studied in a clinical trial.

ATTACHMENT: Wound Product Quality Microbiology

Because a wound represents a breach in the body's natural barrier to microbial invasion, the final formulation of topical products used for the treatment of wounds or burns should be sterile to avoid introducing exogenous microorganisms. Guidance on validation of the manufacture of sterile products can be found in the FDA's *Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products* (November 1994). Methods for performing sterility tests on drug products are currently found in USP 23 Supplement 8 ⟨71⟩ "Sterility Tests."

To avoid contamination of a sterile product, it is preferable for wound products to be packaged in single-use containers. However, if packaged in multi-use containers, wound products should either include a preservative system or possess innate anti-microbial activity. Anti-microbial preservatives should not be used as a substitute for good manufacturing practices. The anti-microbial activity of the product, with (or without) a preservative system, should be demonstrated by performing a microbial challenge test such as the Antimicrobial Effectiveness Test USP 24 Supplement 8 ⟨51⟩. The minimum acceptable limit for the content of preservatives in a product should be demonstrated as microbiologically effective by performing a microbial challenge test of the formulation with an amount of preservative less than or equal to the minimum amount specified as acceptable. For the purpose of application approval, stability data on pilot-scale batches should include results from microbial challenge studies performed on the product at appropriate intervals. Typically, microbial challenge studies are conducted initially, annually, and at expiration. Chemical assays of preservative content should also be performed at all test points. Upon demonstration of the anti-microbial effectiveness of the minimum specified preservative concentration, chemical assays of the preservative may be sufficient to demonstrate the maintenance of adequate anti-microbial activity for annual batches placed into stability testing. For biological products, testing should be done to ensure that the preservative does not compromise biological activity.

Some products cannot withstand sterilization processes because they degrade when heated or irradiated, and

they are not filterable. If a wound product cannot be manufactured to be sterile, it should have a very low bioburden (e.g., ≤10 cfu/g or mL). Bioburden testing should be performed according to a validated test procedure such as USP 23 ⟨61⟩ "Microbial Limit Tests" at appropriate, defined time points during stability studies. Additionally, bioburden testing should include identification of recovered microorganisms to exclude potentially deleterious organisms.

Standards for validation of sterilization of medical devices

ISO 11137:1995 Sterilization of health care products — Requirements for validation and routine control — radiation sterilization
ISO 11135:1994 Medical Devices — Validation and routine control of ethylene oxide sterilization
ISO 11134:1994 Sterilization of health care products — Requirements for validation and routine control — Industrial moist heat sterilization (available in English only)

FOOTNOTES

1. This guidance has been prepared by the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER), and the Center for Devices and Radiological Health (CDRH) at the Food and Drug Administration (FDA). This guidance document represents the Agency's current thinking on developing treatment for chronic cutaneous ulcers and burn wounds. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes, regulations, or both.
2. This document applies only to those medical devices for which clinical studies are required.
3. In 1998, the Agency published a series of draft guidances on developing drugs to treat antimicrobials. Two of those guidances may be of interest: *Developing Antimicrobial Drugs — General Consideration for Clinical Trials* (July 1998) and *Uncomplicated and Complicated Skin and Skin Structure Infections— Developing Anitmicrobial Drugs for Treatment* (July 1998). Once these guidances have been finalized, they will reflect the Agency's views on developing antimicrobial drug products.
4. General guidance for preclinical testing of drugs and biologics can be found in recent International Conference on Harmonisation (ICH) documents, including *M3 Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* (November 1997) and *S6 Pre-Clinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (November 1997).

   For devices, general guidance for assessing preclinical safety can be found in *Blue Book Memorandum #G95-1 Use of International Standard ISO-10993 and Biological Evaluation of Medical Devices Part-1: Evaluation and Testing* (May 1995). See also the draft *Guidance for the Preparation of an IDE Submission for an Interactive Wound and Burn Dressing* , which was published on (April 1995) and is being finalized.
5. Guidance for drug carcinogenicity studies can be found in the ICH documents entitled, *S1A The Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals* (March 1996) and *S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals* (March 1995), Addendum (July 1997).
6. General guidance on preclinical study designs can be found in the ICH document *S5A Detection of Toxicity to Reproduction for Medicinal Products* (September 1994).
7. Further guidance is available in the following ICH documents: *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals* (November 1997) and *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals* (November 1997). The ICH document *S5A Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (September 1994) provides further discussion regarding biological products.
8. General guidance on this topic can be found in ICH documents *E8 General Considerations for Clinical Trials* (December 1997) and *E9 Statistical Principals for Clinical Trials* (September 1998). A draft guidance, *E10 Choice of Control Group in Clinical Trials*, also was published on this topic in September 1999; once finalized, it will reflect the Agency's thinking on clinical trial considerations.
9. As noted under Claims (footnote 3), the Agency published in July 1998 a series of draft guidances on drugs to treat antimicrobials, including uncomplicated and complicated skin infections. These guidances currently are being finalized.
10. General guidance also is available about data analyses, for example *ICH E9 Statistical Principles for Clinical Trials* (September 1998).